Genetics and Molecular Research

# Levels of mannose-binding lectin in individuals with visceral leishmaniasis in the northeast region of Brazil

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Genet. Mol. Res. 14 (4): 19094-19101 (2015)
Received June 24, 2015
Accepted September 11, 2015
Published December 29, 2015
DOI http://dx.doi.org/10.4238/2015.December.29.18

**ABSTRACT.** Visceral leishmaniasis (VL) is one of the seven priority endemic diseases in the world. The clinical outcome of many infections is not only dependent on the pathogenic organism, but also on the genetic variability of the host susceptibility to infection. Mannose-binding lectin (MBL) is a

 

protein that plays an important role in the innate immune system. The aim of this study was to compare the serum levels of MBL between healthy controls and carriers of VL. The VL cases were recruited randomly from the main hospitals and referral outpatient clinics for VL in São Luís, and from home visits. Determination of MBL protein levels was performed by enzyme-linked immunosorbent assay. Of the 161 patients with VL and the 161 healthy controls, 60.9 and 67.1% had high levels of MBL, respectively. There was no significant difference in MBL levels between cases and controls. Low socioeconomic status and living conditions are conducive to the occurrence of VL. Owing to the small number of existing studies, it is extremely important to conduct further studies on MBL levels and susceptibility to VL, especially in regions where the disease is endemic, such as Maranhão, Brazil.

**Key words:** Visceral leishmaniasis; Mannose-biding lectin; Serum concentration

## INTRODUCTION

Visceral leishmaniasis (VL), also known as kala-azar, is an important public health problem and is considered to be one of the world's seven priority endemic diseases. It is a chronic, severe, and highly lethal disease when ignored or inadequately treated (WHO, 2009).

The host inability to developan effective immune response to *Leishmania* infection is associated with several aspects of cellular immunity such as differentiation of lymphocytes, antigen presentation, cytokine production, and the activation mechanisms of macrophage lysis (Ghalibet al., 1993; Karp et al., 1993). Thus, one of the main characteristics of innate immune response deficiency is the interaction between the molecular patterns associated with the pathogen and receptors present on host cells known as pattern recognition receptors (PRR). Some PRRs, such as the mannose-binding lectin (MBL) protein, play an important role in innate immunity (Turner, 2003).

MBL plays an important role in the first line of host defense against pathogens (Super et al., 1989; Turner, 2003). It is a serum protein that belongs to the collecting family; it is mainly synthesized in the liver by hepatocytes and is released into the blood stream (Ezekowitz, 1998). However, research conducted on mice has shown that MBL can also be synthesized by other organs such as the heart, brain, and kidneys (Dumestre-Perard et al., 2002). Furthermore, this protein has the ability to recognize and bind to carbohydrates on the surface of a wide variety of microorganisms such as Gram-positive and Gram-negative bacteria, viruses, fungi, and protozoa (Fraser et al., 1998; Worthley et al., 2005).

The association between MBL and human diseases has been investigated in recent years because of the increasing evidence that correlates genetic variation and susceptibility to some types of infections and autoimmune, metabolic, and cardiovascular diseases (Rector et al., 2001; Roy et al., 2002; Seibold et al., 2004; Fidler et al., 2004; Eisen et al., 2006; Garred et al., 2006; Eisen et al., 2008).

MBL deficiency in human serum has been associated with increased susceptibility to several infections (Lau et al., 1996; Garred et al., 1997; Summerfield et al., 1997; Davies et al.,1997; Graudal et al., 1998; Koch et al., 2001). Infection by VL may be influenced directly by the level of MBL. Furthermore, phagocytosis of promastigotes opsonized with this protein induces the secretion of a higher concentration of TNF-α and IL-6 by human monocytes, demonstrating that MBL may modulate the clinical course and the function of infected monocytes (Santos et al., 2001).

Thus, the present study evaluated these rum levels of MBL protein in individuals with VL and in healthy controls in the regions of São Luís, Maranhão, Brazil, where the disease is endemic.

## MATERIAL AND METHODS

We performed an analytical, cross-sectional study between January 2010 and December 2012 on 322 individuals who lived in regions of São Luís, Maranhão, where VL was endemic.

### Sample

The sample comprised a group of 161 unrelated individuals of both genders (1-50 years of age, 5.1 ± 7.6) with VL (cases), and 161 unrelated healthy subjects of both genders (1-74 years of age, 29.1 ± 13.5) who were not infected by *Leishmania* sp and lived in the same geographic region as the cases (controls). The VL cases were recruited randomly from the main hospitals and referral outpatient clinics for VL in São Luís, and from home visits by searching the records of the National Health Foundation (FUNASA).

The individuals were classified according to the presence or absence of clinical forms as follows: (a) healthy/control/not infected-individuals who had no clinical manifestations of VL and who were negative to anti-*Leishmania* antibodies according to an enzyme-linked immunosorbent assay (ELISA); and (b) VL-symptomatic individuals (sick)-individuals who showed a clinical-laboratory profile of the disease (Brasil, 2009), and who were positive for a parasitological bone marrow aspirate examination for *Leishmania* sp amastigotes forms.

### Quantification of MBL serum levels

Data collection was performed using a questionnaire that identified individuals, epidemiological data, clinical characteristics, and medical examinations. Peripheral blood (5 µL) was used for quantification of MBL serum levels by ELISA (MBL Oligomer ELISA Kit, Copenhagen S, Dinamarca), according to the manufacturer instructions. Levels of MBL above 1000 ng/mL were considered high, 100-1000 ng/mL were considered intermediate, and under 100 ng/mL were considered deficient or low.

### Statistical analysis

Data were analyzed using the SPSS software for Windows 17.0 (2007). To evaluate the effects of group and gender on MBL variables we used the nonparametric Mann-Whitney test. A Spearman correlation was performed todetermine if MBL levelwas relatedto age. Results were considered statistically significant when the P value was not more than 5% ($P \leq 0.05$).

### Ethics

All participating individuals freely provided written informed consent before the collection of samples. The research was approved by the Research Ethics Committee of the University Hospital, Federal University of Maranhão-CEP-HUFMA, with protocol number 005613/2009-80.

## RESULTS

Of the VL cases that participated in this study, 50.3% were female and under 10 years of age (89.4%). Concerning extent of education, 14.3% had studied for less than 8 years. In reference to the monthly income and residence, 47.2% received less than one minimum wage and 56.52% lived in houses made of brick (Table 1).

**Table 1.** Social demographic characteristics of visceral leishmaniasis (VL) cases and healthy controls.

| | Cases | | Controls | |
|---|---|---|---|---|
| | N | % | N | % |
| Gender | | | | |
| Female | 81 | 50.3 | 130 | 80.7 |
| Male | 80 | 49.7 | 31 | 19.3 |
| Age group (years) | | | | |
| <10 | 144 | 89.4 | 6 | 3.7 |
| 11-20 | 8 | 5.0 | 27 | 16.8 |
| 21-30 | 6 | 3.8 | 81 | 50.3 |
| 31-40 | 1 | 0.6 | 24 | 14.9 |
| >40 | 2 | 1.2 | 23 | 14.3 |
| Education level (years) | | | | |
| No education | 3 | 1.9 | 5 | 3.1 |
| ≤8 | 23 | 14.3 | 50 | 31.0 |
| ≥8 | 16 | 10.0 | 101 | 62.7 |
| Not applicable | 127 | 78.9 | 5 | 3.1 |
| Family income (minimum age) | | | | |
| <1 | 76 | 47.2 | 38 | 23.6 |
| 1-2 | 67 | 41.6 | 59 | 36.6 |
| 2-4 | 16 | 9.9 | 40 | 24.8 |
| >4 | - | - | 21 | 13.0 |
| Does not know | 2 | 1.2 | 3 | 2.0 |
| Dwelling type | | | | |
| Brick | 91 | 56.52 | 142 | 88.20 |
| Thatch | 12 | 7.45 | 1 | 0.62 |
| Wood | 47 | 29.20 | 16 | 9.93 |
| Adobe | 7 | 4.35 | - | - |
| Others | 4 | 2.48 | 2 | 1.25 |
| Total | **161** | **100** | **161** | **100** |

Among the 161 unrelated individuals with VL, 107 were hospitalized and 54 had a history of VL.Of the 107 hospitalized individuals, 65 had high levels of MBL, 36 had low levels, and there were six with intermediate levels of serum MBL. Concerning those with a history of VL, 33 had high levels of MBL, 18 had low levels, and there were three with intermediate MBL serum levels (Figure 1).

There was no statistically significant difference in the serum levels of MBL among the carriers of VLand the controls (P = 0.423) andno relation to gender was found (P = 0.326). There was no difference regarding age (P = 0.304) (Table 2). No significant correlation was found between the age of the individual and the level of MBL (r = 0.009; P = 0.876).

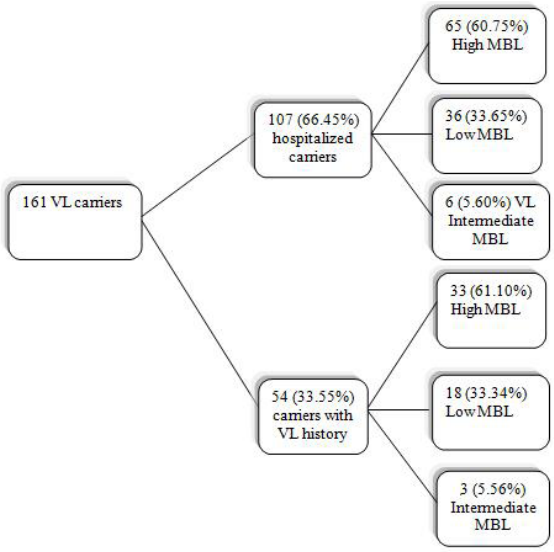


**Figure 1.** Distribution of individuals with visceral leishmaniasis (VL) and serum levels of mannose-binding lectin (MBL).

**Table 2.** Comparison of the levels of mannose-biding lectin (MBL) between groups and between genders using the Mann-Whitney test. Subjects were recruited from São Luís, Maranhão, between January 2010 and December 2012.

| Variable | N | MBL (ng/mL) | P value |
|---|---|---|---|
| | | Mean ± SD | |
| Group | | | |
| Controls | 161 | 18174.1 ± 14099.3 | 0.423 |
| Carriers of VL | 161 | 31884.8 ± 17192.5 | |
| Gender | | | |
| Male | 111 | 38139.0 ± 206792.9 | 0.326 |
| Female | 211 | 18133.0 ± 14336.2 | |

VL = visceral leishmaniasis.

## DISCUSSION

To the best of our knowledge, this is the first study to investigate the levels of MBL protein in individuals with VL in comparison with healthy controlsin regions where the disease is endemic in São Luís, Maranhão, Brazil.

In carriers of VL, no statistically significant difference was found in the level of MBL between genders in our study. However, other studies have shown differences in the level of MBL between genders in those infected with VL. After puberty, teenagers and adult males are among the most affected by VL owing to hormonal fluctuations that may influence susceptibility to the disease (Jeronimo et al., 2007; Nylén et al., 2007).

However, about 90% of cases occur in individuals of less than 10 years of age. Studies have shown that children are more affected by VL than adults, and those under 5 years of age are most susceptible to the disease (Badaró et al.,1986; Maia-Elkhoury et al., 2008; Brasil, 2009), possibly owing to the high rates of nutritional deficiency and the consequent low resistance to disease

in Maranhão, which is considered one of the poorest states in Brazil (IBGE, 2007).

In this study, we noticed the low socioeconomic status among the individuals with VL. According to Nascimento et al. (1996), the ideal environment for VL establishment is mainly low socio economic status, affecting human settlements that live in poor housing conditions.

There was no statistically significant difference in the serum levels of MBL among the carriers of VL and the controls (P = 0.423). MBL deficiency (serum concentration below 100 ng/mL) is associated with severity and susceptibility to infectious diseases (Eisen et al., 2006). However, high MBL concentrations may favor infection by some intracellular parasites such as *Leishmania*, which uses C3 opsonization and its receptor to enter the host cells (Garred et al.,1994).Thus, mechanisms that decrease complement activation may hinder the entry of the parasites into the cells (Santosetal., 2001; Bonar et al., 2004).

The importance of MBL protein in innate immunity has been shown by studies that associate deficiency of MBL with susceptibility to several infectious diseases, mainly extracellular pathogens, and particularly the microorganisms that cause acute infection in childhood (Garred et al.,1995; Garred et al., 1997; Koch et al., 2001; Eisen et al., 2006). Conversely, some evidence suggests that there is a direct association between MBL levels and the risk of infection by some intracellular parasites such as *Leishmania*, although as yet there is no scientific consensus. This is mainly owing to the lack of studies that have sought to elucidate the relationship between MBL and VL.

A study in the northeast region of Brazil found that serum levels of MBL protein are significantly associated with the risk of developing VL within the limits of opsonization higher than 500 ng/mL (Alonso, 2007). The same study also showed that low levels of MBL protein may protect against the progression of VL infection. The author emphasized that in areas of high transmission of the disease there must be epidemiological interventions along with clinical care. Furthermore, the author agreed with the concept that MBL is a “double-edged sword” and that intermediate levels of this protein may be the most desirable phenotype for innate protection against a variety of pathogens (Alonso, 2007). Santos et al. (2001) likewise reported that MBL levels are significantly higherin individuals with VL history than in asymptomatic infected VL individuals. Hamdi et al. (2013) also showed that MBL deficiency decreases the risk of developing VL.

It is noteworthy that of the 161 VL patients evaluated, 107 (66.45%) were admitted with clinical complications. Of these, 65 (60.75%) had high serum level sof MBL protein, 36 (33.65%) had low levels, and 6 (5.60%) had intermediate levels. The remaining 54 individuals were those with a history of VL: 33 (61.10%) with high serum levels of MBL, 18 (33.34%) with low levels, and 3 (5.56%) with intermediate levels. Alonso (2007) reported that high levels of MBL are more frequent among subjects with VL than among asymptomatic infected individuals, and are even more frequent among patients with clinical complications.

Owing to the lack of studies, more research into MBL and susceptibility to VL in endemic regions is necessary in order to increase our knowledge of the infection dynamics of this important public health problem.

## Conflicts of interest

The authors declare no conflict of interest.

## ACKNOWLEDGMENTS

The authors thank FAPEMA for its financial support. The authors also thank the Labora-

tory of Immuno-Physiologyatthe Federal University of Maranhão, the Center of Clinical Research (CEPEC) at the University Hospital of the Federal University of Maranhão, the Laboratory of Genetic Studies and Histocompatibility (LEGH) at the University Hospital of the Federal University of Maranhão, and the Laboratory of Mycology at the University Center of Maranhão.

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